Studies on the resistance of groundnut to *Meloidogyne* sp. in Senegal

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ABSTRACT

Inoculation experiments with different Meloidogyne *isolates from Senegal on groundnut have shown that this crop is highly resistant to these nematodes. Histological observations showed that root tissues react hypersensitively resulting in necrosis of invaded cells.* Meloidogyne *seriously damages groundnut roots if large numbers of juveniles are present in the soil. In soil cropped with groundnut, the numbers of* Meloidogyne *decrease very rapidly in comparison with soil without vegetation containing the same number of nematodes. The dangers are discussed of selecting* Meloidogyne *populations capable of attacking groundnut as a result of the incorporation of this crop in rotations intended to control these nematodes in heavily infested soils. It seems prudent to use groundnut in rotations as a preventive and not as a curative control method for* Meloidogyne.

RÉSUMÉ

Des expériences d'inoculation sur arachide, effectuées avec des Meloidogyne *provenant de diverses régions du Sénégal ont montré que cette plante est très résistante à ces nématodes.*

Des études histologiques ont permis d'observer une réaction d'hypersensitivité des tissus des racines, aboutissant à la nécrose des cellules attaquées.

Si un grand nombre de juvéniles de Meloidogyne *sont présents dans le sol, les racines de l'arachide seront gravement endommagées. Dans un sol cultivé en arachide, le nombre de* Meloidogyne *diminue très rapidement en comparaison avec un sol sans végétation contenant le même nombre de nématodes.*

Il est discuté du risque de sélectionner des populations de Meloidogyne *capables d'attaquer l'arachide à la suite de l'utilisation de cette plante dans des rotations destinées à lutter contre ces nématodes dans des sols très infestés. Il semble prudent d'utiliser l'arachide dans de telles rotations en tant que méthode de lutte préventive mais non curative contre* Meloidogyne.

INTRODUCTION

Recently, it has been shown that in Senegal groundnut actively reduces the number of *Meloidogyne* when grown in heavily infested soils (NETSCHER, 1974). This is due to the fact that the nematodes once they have penetrated the roots are trapped and subsequently die.

In order to obtain more information concerning this phenomenon a series of experiments was carried out. They include comparison of the behaviour of different isolates of *Meloidogyne* towards groundnut, varietal comparisons, determination of the tolerance level of groundnut, histological observations and a study of the decrease in the number of nematodes under the influence of groundnut.

MATERIAL AND METHODS

Different isolates of *Meloidogyne* were inoculated to three-weeks old groundnut seedlings (var. 55-437) growing in 250 cm³ pots filled with sterile soil at a rate of 1,000 juveniles per pot. Two months after inoculation the roots were carefully washed and placed in a Seinhorst mistifier (SEINHORST, 1950) during one week in order to extract juveniles and males of *Meloidogyne* which had developed.

Ten pots of each of 7 varieties of groundnut (I.R.A.T. numbers 28.206, 29.127, 47.16, 48.115, 55.437, 57.422, and 69.102) were inoculated with 1000 juveniles of *Meloidogyne* cf. *arenaria*. Four months after inoculation the root systems of all plants were placed in a Seinhorst mistifier to determine the number of males and juveniles present in each variety.

Groundnut (var. IRAT 55.437) was sown in 250 cm³ pots containing sterile soil inoculated respectively with 0, 100, 200, 400, 800, 1.600, and 3.200 juveniles of *Meloidogyne* cf. *arenaria*. Each treatment was repeated 10 times. The same experiment was repeated with the same *Meloidogyne* population using pots of 100 cm³ and inoculum levels of 0, 400, 800, 1.600, 3.200, 6.400, and 10.000 juveniles. Ten days after sowing the percentage of germination and the weight of each plant was determined.

Roots of badly-growing groundnuts sown in pots containing several thousands of *Meloidogyne* juveniles were fixed for at least 48 hours in FAA. Roots were imbedded in paraffin, sectioned at a thickness of 15 µm with a Leitz Minot microtome 1212 and stained with safranin and fast green. Photographs were taken with a Leitz « Orthomat » photomicroscope. For routine observations roots were stained in boiling acid fuchsin lacto-phenol for three minutes and flattened between two microscope slides and examined under a dissecting microscope.

Two-week old seedlings growing in 250 cm³ pots filled with sterile soil were inoculated with 1000 juveniles of *M. javanica*. As a control the same number of nematodes was inoculated to pots filled with sterile soil without groundnut plants. Twelve hours after inoculation the number of juveniles present in the soil of 5 controls was determined by extracting the soil in a Seinhorst elutriator (SEINHORST, 1962). From then on, soil of 5 pots each of groundnut and control soil were extracted at intervals of three days and the number of *Meloidogyne* juveniles determined.

RESULTS

Of the several isolates tested on groundnut (var. 55-437) a few exhibited a very limited reproduction as is shown in Table I.

TABLE I

NUMBERS OF *MELOIDOGYNE* RECOVERED TWO MONTHS AFTER INOCULATION OF 1000 JUVENILES FROM ROOTS OF VARIETY 55-437

Sample number	*Origin*	*Species*[1]	*Number of juveniles*	*Number of males*
10 803	Cape Point (Gambia)	*inc.*[2] + *jav.*	0	0
11 317	Kolobane (Dakar)	*inc.* + *ar.*	0	0
11 320	Thiaroye (Dakar)	*jav.* + *inc.*	0	0
11 313	Grand Yoff (Dakar)	—	0	0
11 327	Thiaroye/mer	*jav.*	0	0
11 318	Camberene	—	0	0
11 331	Yeumbeul	*jav.*	0	0
11 332	»	—	13	0
11 334	Malika	—	19	0
11 341	Savoigne	cf. *ar.*	0	0
11 351	Mbaye Khol	sp.	0	0
11 352	Rao	*jav.* + *inc.*	0	0
11 357	Hann (Dakar)	—	0	0
11 359	Thiaroye	—	0	0
11 575	Kirene	*inc.*	0	0
11 622	Sebikotane	*jav.*	30	0
12 097	Dakar	*inc.* + *jav.*	13	0
12 099	Yeumbeul	*inc.*	6	0

1 Species determined after perineal patterns.

2 *ar.* = *Meloidogyne arenaria* ; *inc.* = *Meloidogyne incognita*. *jav.* = *Meloidogyne javanica*.

The few juveniles obtained failed to reproduce when reinoculated to groundnut.

Table II summarizes the results of inoculations of an isolate of *M.* cf. *arenaria* from a heavily infested tomato field to seven different varieties of groundnut.

TABLE II

NUMBER OF *MELOIDOGYNE* CF *ARENARIA* RECOVERED FROM TEN ROOT SYSTEMS OF DIFFERENT GROUNDNUT VARIETIES FOUR MONTHS AFTER INOCULATION WITH 1000 JUVENILES PER PLANT

Variety	*Number of juveniles*	*Number of males*
28 206	1	0
29 127	0	0
47 16	12	0
48 115	0	0
55 437	0	0
57 422	0	0
69 102	160	0

The 160 juveniles obtained on 69-120 were inoculated to susceptible tomato but failed to reproduce. In fact more isolates are being tested to make sure that this variety does not possess a certain tolerance to *Meloidogyne* species from Senegal.

As it was observed that groundnuts sown in soils heavily infested with root-knot have difficulties in establishing themselves, a histological study was made of roots growing in infested soil. Examination of longitudinal and cross sections of roots showed that juveniles penetrate roots in great numbers, causing a necrosis of cells in the vicinity of the nematodes (Fig. 1). Sometimes the nematodes have incompletely penetrated and stay half embedded in the roots (Fig. 2). Macroscopic symptoms are browning of tap roots and lateral roots causing a retardation in growth (Fig. 5 and 7). Sometimes the germinating tap root is damaged to such an extent that new ones are formed. In very severe attacks the tap root becomes completely necrotic and all growth stops (Fig. 6).

In the case of a slight attack nematodes do not necessarily cause surrounding root cells to die (Fig. 3). In this case the juveniles do not develop and are enclosed by the tissue without apparently doing any damage. Figure 4 shows such a juvenile incorporated in differentiating vascular tissue.

In order to determine at what level *Meloidogyne* seriously hampers root development of the soil, pots of 250 cm³ sown with groundnut were inoculated with 0, 200, 400, 800, 1.600 and 3.200 juveniles of *M.* cf. *arenaria*. As three weeks after sowing no noticeable difference between any of the treatments could be observed, a second experiment was set up using pots of 100 cm³ with a higher inoculum level. Table III

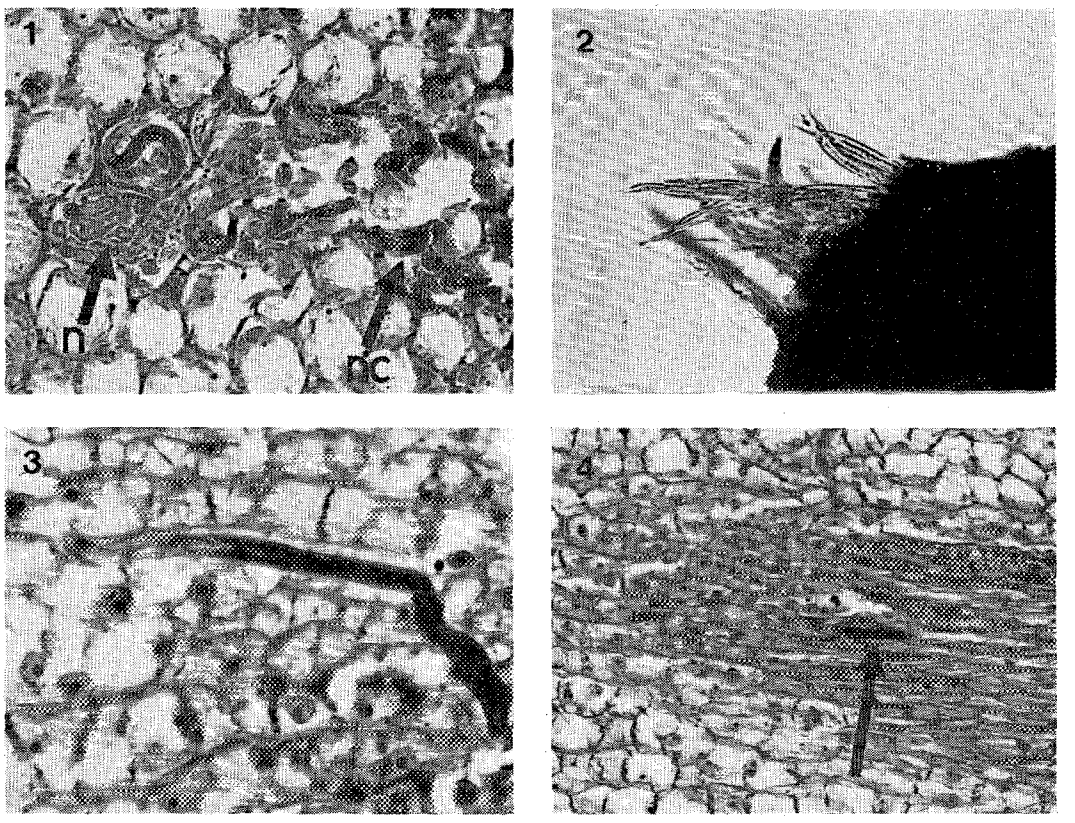

Fig. 1. — Juveniles of *Meloidogyne* which have penetrated in the cortical tissue of groundnut roots (n) ; and accompanying necrosis (nc). Fig. 2. — Juveniles of *Meloidogyne* which have partially penetrated root tip. Fig. 3. — Juveniles of *Meloidogyne* enclosed in root-tissue without any apparent cell-necrosis. Fig. 4. — Juvenile of *Meloidogyne* (arrow) envelopped in differentiating vascular tissue. Figures 1, 3 and 4 stained with saffranin and fast green ; fig. 2 stained with acid fuchsine lactophenol

TABLE III

MEAN WEIGHT OF GERMINATED SEEDLINGS AND PERCENTAGE OF GERMINATION OF GROUNDNUT INOCULATED WITH DIFFERENT NUMBERS OF *MELOIDOGYNE* CF *ARENARIA*

Number of juveniles	*Percentage of germination*[1]	*Mean weight (gr)*
0	100	3,2
400	90	2,7
800	100	2,6
1.600	80	2,7
3 200	70	1,8
6 400	80	1,7
10.000	70	1,3

[1] 10 seeds were planted per treatment.

shows the difference in weight of entire seedlings 10 days after sowing.

A prononced difference is observed between growth at population levels above and below 3.200 juveniles per 100 cm^3, showing that at this and higher levels groundnut development is seriously hampered, figure 5 illustrates this harmful effect very cleraly.

In order to estimate the quantitative effect of groundnut on *Meloidogyne* populations, an experiment was carried out in which the number of juveniles recovered from soil with groundnut was compared with that of soil without vegetation. Figure 8 illustrates the difference in the rate of decrease of *Meloidogyne* juveniles from soil of pots with and without groundnut, previously inoculated with 1.000 nematodes. During the first 12 hours after inoculation more than 50% of the nematodes died. In the controls the number of juveniles slowly declined afterwards whereas in pots with groundnut a sharp decrease took place during the first three days of the experiment probably due to the trapping effect of the roots. Though root necrosis was noted, no serious damage was observed.

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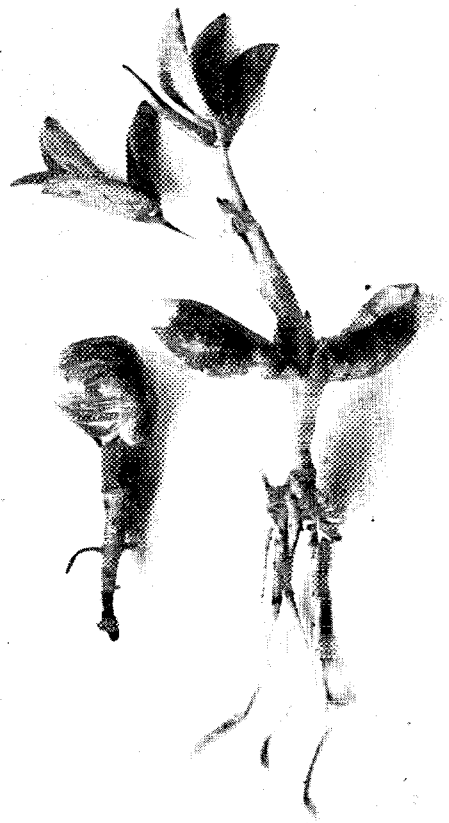

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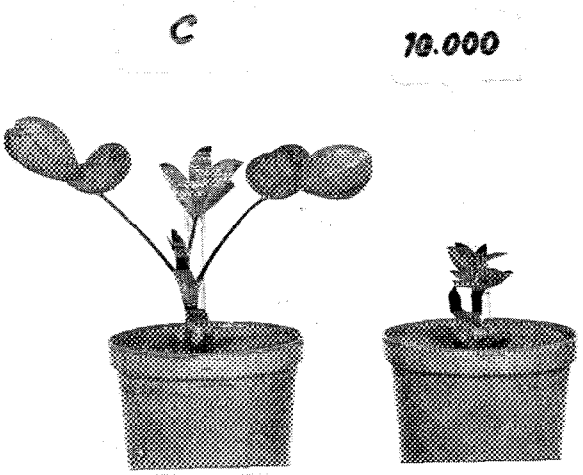

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Fig. 5. — Groundnut seedlings inoculated with (left) 1600 and (right) 3200 juveniles of *Meloidogyne*. Fig. 6. — Groundnut seedlings attacked by juveniles of *Meloidogyne* ; left : growth completely suppressed after death of tap root ; right : tap root replaced by numerous adventitious roots. Fig. 7. — Effect of *Meloidogyne* on growth of groundnut seedlings; left : control; right: seedling inoculated with 10 000 juveniles

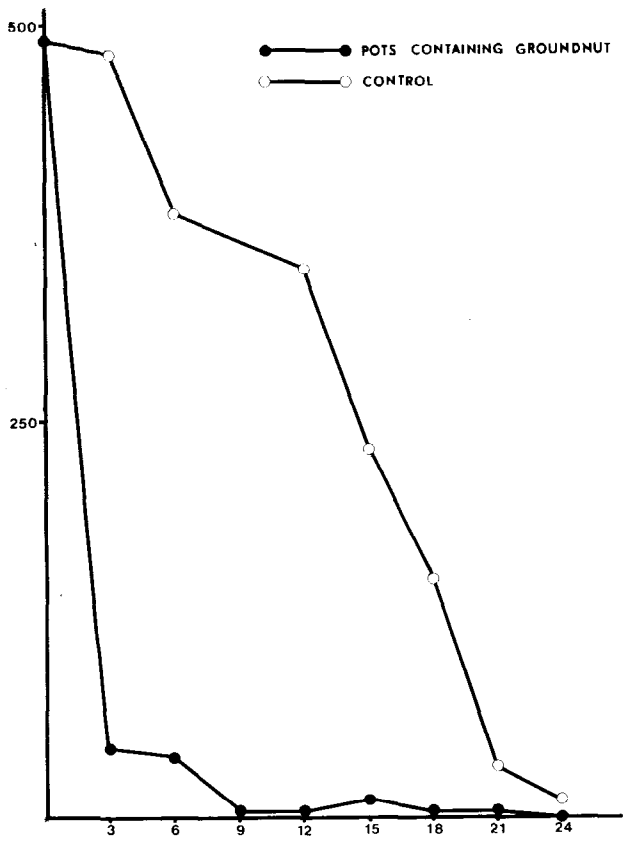

Fig. 8. — Decrease in numbers of juveniles of *Meloidogyne* under influence of groundnut
Abcissa : time in days ; ordinate : mean number of juveniles recovered from five pots

DISCUSSION

The results of the experiments reported here clearly show that groundnut has a strong repressive effect on the *Meloidogyne* populations tested, due to a hypersensitive reaction of the root tissue invaded by the nematodes. Such hypersensitive reactions have been observed with root-knot resistant varieties of plants like soybean, tomato, and sweetpotato (DROPKIN & NELSON, 1960 ; RIGGS & WINSTEAD, 1959 ; GIAMALVA *et al.*, 1963), but indications of this phenomenon on groundnut have been controversial.

SASSER (1954) stated that groundnut is susceptible to *Meloidogyne arenaria* and *M. hapla*, but resistant to *M. incognita* and *M. javanica*. Though he observed juveniles of *M. incognita* in groundnut roots he did not mention necrosis of root tissue. MINTON (1963) found that an isolate of *M. arenaria* from Alabama heavily attacked groundnut whereas another from North Carolina attacked this crop only slightly. Though root necrosis was caused by both isolates, the North Carolina population induced a more pronounced necrosis. MARTIN (1956, 1958) occasionally observed reproduction of *M. arenaria* and *M. javanica* on groundnut in East Africa, though heavy attacks were not observed. In early stages of attack by *M. javanica*, MARTIN noted that quite a number of the root tips showed evidence of rot; in the light of the observations of the present study it seems likely that this root rot was actually root necrosis caused by invading nematodes. PEACOCK (1957) however, using various isolates of *Meloidogyne* stained by the same techniques as used in this study failed to notice the presence in the roots of any of these nematodes.

The differences mentioned above may be easily explained if one assumes that those workers who did not observe root necrosis examined well established root systems that had been invaded during the early stages of development of the plant. Nematodes invading the root tips caused so much damage that the roots died. The dead tissues containing the invading juveniles of *Meloidogyne* quickly disintegrated in the soil and were replaced by newly-formed roots which were either healthy or only slightly attacked because the bulk of the parasites had been trapped before. In fact on roots of plants inoculated four months before examination, little or no necrosis was found while recently inoculated plants showed the strong symptoms such as shown in Figures 5 and 7.

Strong indications have been obtained that a previous crop of groundnut grown in a soil heavily infested by *Meloidogyne* has a beneficial effect on a subsequent susceptible crop. Unfortunately a danger exists in that an adaptation of *Meloidogyne* to groundnut might take place. As mentioned above, attacks of *M. arenaria* and *M. javanica* have been recorded on groundnut. In fact, *M. arenaria*, often called « the peanut root-knot nematode» and considered a serious parasite in the U.S. is one of the *Meloidogyne* species frequently encountered in the tropics (the others being *M. incognita* and *M. javanica*). This species has been found throughout West Africa (ADDOH, 1970 ; CAVENESS, 1965 ; NETSCHER, 1970), though no apparent damage on groundnut has been reported.

The isolates tested in this study (Table I) only represent an infinitissimal proportion of the existing populations and taking into account the great variability of *Meloidogyne*, there is no guarantee that populations agressive towards groundnut should not exist. Recent work in Florida (KIRBY *et al.*, 1975) shows that these considerations are far from hypothetical : from 6 populations of *M. arenaria* tested, three were capable of attacking groundnut and three not.

Admitting the possibility that populations agressive towards groundnut might exist, the question may be raised as to why there has never been a root-knot problem on groundnut in Senegal though hundreds of thousands of hectares are grown yearly with this crop. The answer to this question may be that condi-

tions are very unfavourable for the development of *Meloidogyne* in soils regularly cropped with groundnut[1].

Taking these considerations into account the risk of selecting *Meloidogyne* populations capable of attacking groundnut when growing this crop in heavily infested soils in order to control the nematodes is not completely imaginary. However, for the very reasons mentioned above (bad conditions for the development of *Meloidogyne* in groundnut fields) a massive infestation of the soils traditionally cropped with groundnut seems unlikely, especially because the spread of nematodes is very limited in comparison with that of other plant pathogens. Still it is prudent to avoid incorporation of groundnut in crop rotations on heavily infested soils.

Another reason for not growing groundnut in heavily infested fields is to avoid possible failure of the crop because of the insufficient development of the roots. The threshold of 3200 juveniles in 100 cm^3 pots (see table III) corresponds to a level of infestation frequently found in the field. In fact we have observed complete failure of groundnut due to *Meloidogyne* in heavily infested soils. Examination of seedlings inoculated two weeks after sowing with 1000 juveniles showed that roots were not seriously hampered in their growth though several hundreds of nematodes penetrated the roots within a few days (Fig. 8). The ability of groundnut to produce a fairly extensive root system in a short time enables this plant to clean moderately infested fields quite efficiently, provided the roots get a chance to establish themselves.

On the basis of the observations made in this study it seems likely that the use of groundnut in crop rotation may on the one hand prevent the normally unavoidable build-up of root-knot nematodes under the influence of continuous cropping of susceptible plants and on the other hand accomplish the cleaning of slightly infested soils.

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[1] Cowpea, a very susceptible plant, is rarely attacked in Senegal. Like groundnut, the crop is grown once every three years in rotation with millet and sorghum, both poor hosts for root-knot nematodes. Each cropping season of four months is followed by 8 months of drought during which period nematode populations are greatly reduced.